

### **REMARKS**

Claims 1 and 6-11 are pending in the application. Claims 1, 6 and 7 have been amended and claims 2-5 have been cancelled herein. Favorable reconsideration of the application is respectfully requested in view of the amendments to the claims and following comments.

#### ***I. CLAIM REJECTIONS UNDER 35 U.S.C. §103(a)***

Claims 1-4 and 9-11 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Fuji et al. (WO 02/097107) in view of Motoo et al. (JP 11-298058) and Suzaki et al. (Bioconversion of Cellulose to  $\alpha$ -1,4-Glucan, 1983). The Examiner acknowledges that Fuji et al. fails to disclose that a  $\beta$ -1,4-glucan such as cellobiose or celloligosaccharide is used instead of sucrose as the raw material for producing the intermediate G1P, and further fails to disclose that cellobiose phosphorylase (CBP) and cellodextrin phosphorylase are applied on  $\beta$ -1,4-glucans instead of sucrose phosphorylase.

It is the Examiner's position that it would have been obvious to one of ordinary skill in the art to substitute cellobiose or celooligosaccharide of polymerization n and cellobiose phosphorylase and/or cellodextrin phosphorylase for sucrose and sucrose phosphorylase, respectively, to produce  $\alpha$ -1,4-glucans. The Examiner contends that the ordinary artisan would have realized that the reverse reaction taught by Motoo et al. is the same as that employed by Fuji et al. The Examiner further contends that the ordinary artisan would have had a reasonable expectation that cellobiose and CPB or oligocellobiose and CPB and cellodextrin phosphorylase could be substituted for sucrose and sucrose phosphorylase since Suzaki et al. teach that the reverse reaction taught by Fuji et al. reacts in the forward direction to provide amylose.

Claim 1 has been amended to substitute "cellobiose" for " $\beta$ -1,4-glucan". Support for this amendment may be found in the English language specification at page 8, lines 5-7 and at page 81, line 28 to page 92, line 4. Claim 1 has been further amended to recite that the step of reacting the solution to produce the  $\alpha$ -glucan comprises removing glucose produced as a byproduct from the solution simultaneously with production of the  $\alpha$ -glucan. Support for this amendment may be found in the English language specification at page 8,

lines 19-21; page 70, lines 17-32; page 72, line 17 to page 73, line 4; and page 89, line 14 to page 91, line 19. Claims 6 and 7 have been amended to depend directly from claim 1.

The present invention is directed to a method for production of an  $\alpha$ -glucan from a cellobiose, comprising: reacting a solution containing a cellobiose, a primer, a source of phosphoric acid, cellobiose phosphorylase, and  $\alpha$ -1,4-glucan phosphorylase to produce an  $\alpha$ -glucan; wherein the step of reacting the solution to produce the  $\alpha$ -glucan comprises removing glucose produced as a byproduct from said solution simultaneously with production of said  $\alpha$ -glucan.

According to the present method, non-digestible cellulose can be efficiently converted into a digestible food. In particular, removal of glucose produced as a byproduct from the solution simultaneously with production of the  $\alpha$ -glucan remarkably improves the yield of the  $\alpha$ -glucan.

In the present method, two reactions are combined, and two different phosphorylases are combined. In such a method, those skilled in the art could not predict that the reactions can proceed successfully in one reaction solution. Those skilled in the art could not predict the direction of the reactions. This is because complicated reactions are conducted with two types of enzymes which share substrate and product, and the reaction mechanism is quite complex. Therefore, it is common technical knowledge that simple combination of two types of enzymes would not necessarily convert the substrate into a desired product. In view of the common technical knowledge, the claimed method would not have been expected to be successful.

In the present method, glucose produced as a byproduct of  $\alpha$ -glucan production is removed from the solution. By removing glucose, the yield of  $\alpha$ -glucan is improved about 1.4 to 2 times as compared to the reaction in which the produced glucose is not removed. Specifically, the yield of  $\alpha$ -glucan in Example 5-1 of the present specification (not removing glucose) is 32.8%, whereas the yields of  $\alpha$ -glucan in Examples 5-2, 5-3 and 5-4 of the present specification are 45.6, 54.9 and 64.8%, respectively. (See Table 5 on page 90 of the English language specification.)

The improvement of production yield by removal of glucose is a remarkable and unexpected effect obtained by the claimed method, and this effect cannot be obtained by

another reaction system. For example, as shown in Reference Example 2 on page 94, line 23 to page 96, line 5 of the English language specification of the present application, in the reaction system in which sucrose is produced from cellobiose, by removing glucose from the reaction system, the yield of sucrose was not improved significantly.

In the cited references Fuji et al., Motoo et al. and Sasaki et al., improvement of production yield of the product of interest by removing a byproduct is not described. Furthermore, the cited references do not disclose or suggest that removal of glucose proceed the reaction toward the production of  $\alpha$ -glucan, and thus improve the production yield of the  $\alpha$ -glucan. As the Examiner recognized, Fuji et al., Motoo et al. and Sasaki et al. do not teach removing glucose produced as byproducts from the solution simultaneously with the production of the alpha-glucan. Accordingly, Applicants respectfully submit that the combined teachings of Fuji et al. , Motoo et al. and Suzaki et al. do not render the method of claims 1 and 9-11 obvious under 35 U.S.C. §103(a).

Claims 1-11 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Fuji et al. in view of Motoo et al. and Suzaki et al., and further in view of Kitaoka et al. (Carbohydrate-Processing Phosphorolytic Enzymes, 2002) and Ota et al. (JP 10-099098). As discussed above, the Examiner acknowledges that the combined disclosures of Fuji et al., Motoo et al. and Sasaki et al. fail to teach removing glucose produced as a byproduct from the solution simultaneously with the production of the alpha-glucan by reaction with glucose oxidase, mutarotase and catalase. The Examiner asserts that Kitaoka teaches that CBP is inhibited by product inhibition due to the production of G1P and the byproduct, glucose (page 43, left column), and that the ordinary artisan would understand from this disclosure that inhibition of CBP will reduce the yield of the desired product. However, it appears that the Examiner has misinterpreted the disclosure of Kitaoka.

Kitaoka does not teach or suggest that the inhibition of CBP will reduce the yield of the desired product. As Kitaoka describes "[I]n the reverse reaction, substrate inhibition was observed using glucose, glucosamine and 6-deoxyglucose as the acceptors, with glucose displaying the strongest level of inhibition. It was assumed that the phenomenon was caused by the competitive inhibition of the substrate glucose toward glucose-1-

phosphate, the other substrate, namely a 'competitive substrate inhibition' (Fig. 6)." (Kitaoka at page 43, left column, 2<sup>nd</sup> paragraph.)

In view of Fig. 6 on page 44 of Kitaoka, competitive substrate inhibition would be caused by inhibition of the reaction of synthesizing cellobiose by CBP due to occupation of reaction site in the CBP by Glc instead of G-1-P. Thus, it was previously thought that it is important to reduce the concentration of glucose in the reaction solution in order to produce cellobiose. Therefore, it is important to increase the concentration of glucose in the reaction solution in order to degrade cellobiose.

In the present invention, promotion of the reaction of the degradation of cellobiose is important. In view of the disclosure of Kitaoka, those skilled in the art would consider that by inhibition of cellobiose production, degradation of cellobiose which is the reverse reaction of the production would be promoted. Thus, those skilled in the art would add glucose in order to promote the degradation of cellobiose, rather than remove glucose from the reaction.

With regard to Ota et al., the Examiner states that "Ota teaches that trehalose can be measured more accurately by combining trehalose phosphorylase (TP) with enzymes that eliminate the influence of the glucose byproduct as inhibitor of TP". However, it appears that the Examiner has misinterpreted the disclosure of Ota. Ota does not teach or suggest that trehalose phosphorylase (TP) is combined with enzymes that eliminate the influence of the glucose byproduct as inhibitor of TP. Ota does not describe or suggest a method of eliminating a glucose byproduct.

It is noted that the machine translation of Ota, which is referred to by the Examiner, does not contain a translation of the drawings. Therefore, Applicants enclose an English translation of Figures 1(a) and 1(b), attached hereto as Exhibit A.

Ota discloses a simple and accurate method of measuring an amount of trehalose. As shown in Figure 1(b) of Ota, Ota describes that trehalose is degraded by the enzymatic reaction to produce  $\alpha$ -D-glucose (i.e., glucose), the produced  $\alpha$ -D-glucose is converted to  $\beta$ -D-glucose, the produced  $\beta$ -D-glucose is used for production of  $\beta$ -D-glucose- $\delta$ -lactone and  $H_2O_2$ , and the produced  $H_2O_2$  is used for color development. Based on this color development, the amount of trehalose is measured.

Thus, the glucose produced by the enzymatic reaction of Ota is not eliminated but is used for measuring an amount of the trehalose. Therefore, the reaction mixture obtained by the enzymatic reaction must contain only the glucose (and  $\text{H}_2\text{O}_2$ ) derived from trehalose. If the reaction mixture contains glucose (and  $\text{H}_2\text{O}_2$ ) which is not derived from trehalose but which was originally contained in the starting material, the result of the measurement is inaccurate. Therefore, as shown in Figure 1(a), Ota discloses that glucose (and  $\text{H}_2\text{O}_2$ ) which was originally contained in the starting material should be removed before performing the step of producing glucose from trehalose.

In this regard, Ota describes that “the glucose which exists in a sample can serve as an interfering factor from the beginning in the enzymatic quantitation reaction of trehalose” in paragraph [0013], and “[s]ince the hydrogen peroxide generated here also affects the enzymatic quantitation reaction of trehalose, it is changed into water and oxygen by catalase” in paragraph [0014]. These descriptions do not mean that glucose acts as an inhibitor of trehalose phosphorylase. Ota merely describes that the glucose which originally exists in the starting material should not be used and only the glucose derived from trehalose should be used in order to measure an accurate amount of trehalose. That is, the phrase “the glucose which exists in a sample can serve as an interfering factor” in paragraph [0013] does not mean that glucose interferes with the enzymatic reaction, but means that the glucose which originally exists in the sample interferes with the measurement of an accurate amount of trehalose, and therefore only the glucose that is derived from trehalose should be used. Ota does not describe or suggest that an enzymatic reaction is inhibited or interfered by glucose.

Furthermore, Ota does not describe glucose as a byproduct. As shown in Figure 1(b), in the method of Ota, glucose is not a byproduct, but glucose-1-phosphate and  $\beta$ -D-glucose- $\delta$ -lactone are byproducts. The glucose derived from trehalose in the process of Ota is an intermediate product of interest which is used for measuring the amount of trehalose. Therefore, in order to measure an accurate amount of trehalose, the method of Ota employs a step of eliminating glucose (and  $\text{H}_2\text{O}_2$ ) which is not derived from trehalose but which was originally contained in the starting material.

As discussed above, Ota describes that the glucose (and  $H_2O_2$ ) which originally exists in the starting material should be eliminated and that the glucose derived from trehalose should not be eliminated, but should be used for measuring an amount of trehalose. Ota does not describe glucose as a byproduct.

Additionally, the purpose and the effect of the removal of glucose are quite different between the present invention and the method described in Ota. Further, the method described in Ota is not related to the inhibitory effect of glucose. That is, glucose does not inhibit the enzymatic reactions in the method of Ota. Therefore, Ota does not teach or suggest the effect of removal of glucose on production yield.

Furthermore, the other cited references do not disclose or suggest the removal of glucose. The effect of the removal of byproduct glucose would not be expected from the combined teachings of the cited references. In view of the foregoing amendments and remarks, Applicants respectfully request withdrawal of the rejection of claims 1, and 6-11 under 35 U.S.C. §103(a).

## **II. CONCLUSION**

Accordingly, all claims are believed to be allowable and the application is believed to be in condition for allowance. A prompt action to such end is earnestly solicited.

Should the Examiner feel that a telephone interview would be helpful to facilitate favorable prosecution of the above-identified application, the Examiner is invited to contact the undersigned at the telephone number provided below.

Should a petition for an extension of time be necessary for the timely reply to the outstanding Office Action (or if such a petition has been made and an additional extension is necessary), petition is hereby made and the Commissioner is authorized to charge any fees (including additional claim fees) to Deposit Account No. 18-0988.

Respectfully submitted,

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## Exhibit A

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### Partial Translation of Ota (JP Laid-Open Publication No. 10-099098)

[ Figure 1 ]

(a)



(b)

(catalase — inhibition by sodium azide)

